

STAGES IN THE LIFE HISTORY OF A SYMBIOTIC ZOOXANTHELLA
IN PELLETS EXTRUDED BY ITS HOST *AIPTASIA TAGETES*
(DUCH. AND MICH.) (COELENTERATA, ANTHOZOA)

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In 1944 Kawaguti extracted from the coral *Acropora corymbosa* a number of its symbiotic algae and grew these in culture. Observation of these cultures revealed that the zooxanthellae regularly gave rise to motile individuals, and from their characteristics he classified them as being dinoflagellates of the Genus *Gymnodinium*. McLaughlin and Zahl (1957, 1959, 1966) subsequently extracted symbiotic zooxanthellae from a number of coelenterates and produced axenic cultures of these algae. The coelenterates used were the scyphozoan *Cassiopeia sp.*, the alcyonarians *Antillogorgia acerosa* (Passas) and *Plexaurella dichotoma* (Esper), the scleractinians *Cladocora sp.* and *Porites sp.* and the actinian *Condylactis sp.* The algae isolated from all these species appear to be the same insofar as they all gave rise to similar gymnodinoid forms.

Freudenthal (1962), using axenic cultures of zooxanthellae obtained from *Cassiopeia*, also identified this symbiont as being a dinoflagellate and assigned to it the name *Symbiodinium microadriaticum* (Freudenthal). He described the life history of the organism and found it to be a cycle in which the zooxanthellae are encysted stages that alternate with motile ones. Taylor (1971a) later renamed the species *Gymnodinium microadriaticum* (Freudenthal) and in subsequent work (1973a) described it as being the dinoflagellate symbiont most commonly occurring among benthic dwelling hosts. Based on an extended study of this species, both *in situ* and in culture, he further proposed a modified version of Freudenthal's scheme as being a more accurate representation of the life history of that organism (see Fig. 1).

The immature, mature, dividing and degenerate cysts which form stages in the life history of *G. microadriaticum* have all been observed in mucous boli and strings extruded under normal conditions by a variety of coelenterates (Taylor, 1969, 1973a; Goreau, Goreau, Yonge and Neumann, 1970; Reimer, 1971; Yonge, 1973; Trench, 1974). However, with the exception of the dinoflagellate *Amphidinium klebsii* (Kofoid and Swezy) which is known to be both free-living and symbiotic (Taylor, 1971b) and the motile stages of *S.* (= *G.*) *microadriaticum* which were observed by McLaughlin and Zahl (1959) to be released from disintegrating specimens of *Condylactis* kept in an aquarium, dinoflagellate symbionts have not been collected as free-living stages in the environment adjacent to their hosts, although T. F. Goreau (N. I. Goreau, Zoology Department, University of the West Indies, personal communication) had recorded the presence of dumb-bell shaped zoospores among zooxanthellae freshly extracted from *Zoanthus sp.* in 1965.

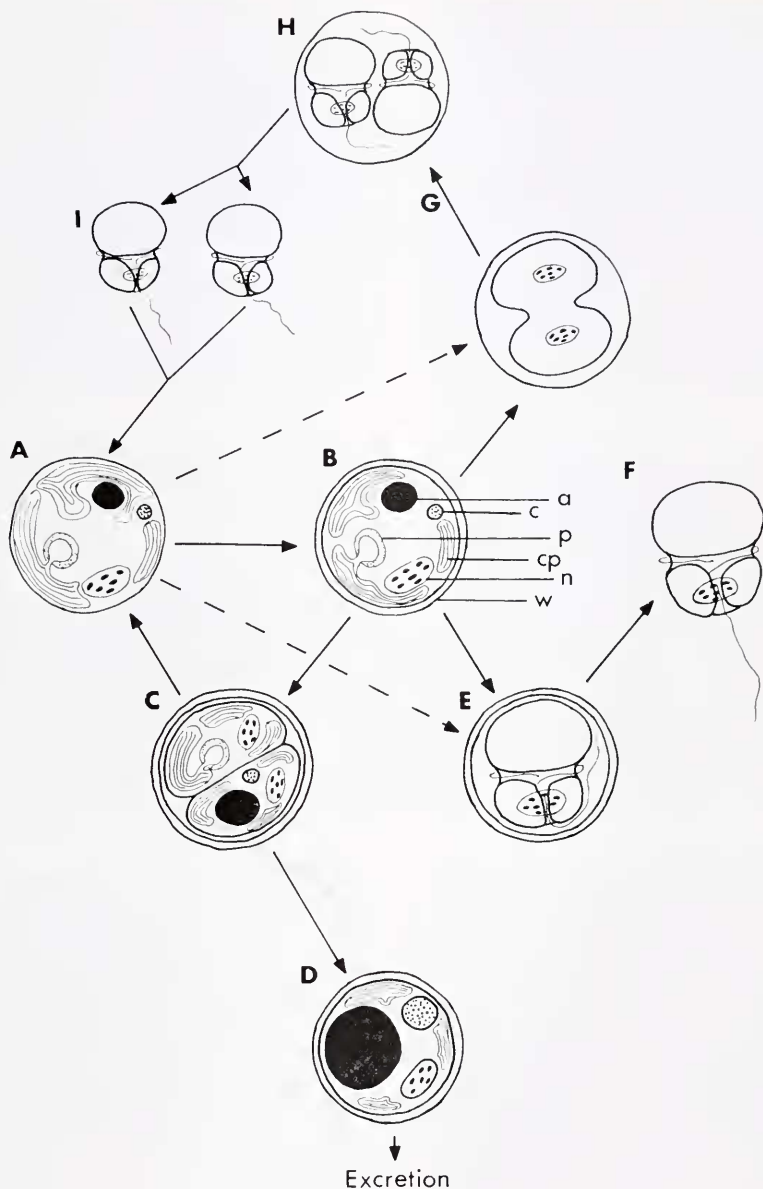


FIGURE 1. Outline of the life history of *Gymnodinium microadriaticum* after Taylor (1973a). (A) shows an immature cyst; (B) a mature cyst; (C) division of a cyst into a physiologically younger cell which reverts to an immature cyst (A), and a physiologically older cell (D) which degenerates and is excreted. (E) shows a zoosporangium; (F) a motile zoospore; (G) and (H) the development of gametes and (I) the release of mature gametes. The accumulation body is represented by (a); calcium oxalate (c); chloroplast (cp); nucleus (n); pyrenoid (p); and cyst wall (w).

This paper reports the observation of a number of stages in the life cycle of the zooxanthella symbiotic with the anemone *Aiptasia tagetes* in material extruded by the host.

OBSERVATIONS

Healthy specimens of *Aiptasia tagetes* kept in the laboratory regularly extrude dark-brown, compact pellets made up almost exclusively of zooxanthellae held together by mucus. These pellets are discharged separately from those containing faecal material (R. D. Steele, unpublished observation). Examination of smears of these algal-containing pellets under the microscope revealed the presence of the following stages in the life cycle of the alga.

Stages which are always observed

Cysts (see Fig. 2A and B). These form the majority of cells in the pellets. They are 5.3 to 11.7 microns in diameter, show a pyrenoid, nucleus, vacuoles with rapidly moving contents and often a reddish-brown accumulation body. Mature cysts all have a thick wall (see Fig. 2B).

Dividing cysts (see Fig. 2C, D, E). The number of these in a pellet varies widely but never exceeds 25 percent of the total. Normally, two closely apposed daughter cells are present within a cyst wall but quite frequently groups of four have been noted (see Fig. 1A). In a number of cases the cyst wall has been lost. This occurs among zooxanthellae in which division is complete and whose daughter cells, though still attached, have developed their own cell walls (see Fig. 1D and E).

Degenerate cysts (see Fig. 2F). These cysts form a small percentage of the total algal content of the pellets. Variable degrees of degeneracy are found, but in general the degenerate zooxanthella is characterized by the presence of a large accumulation body, an increase in number and size of cytoplasmic inclusions, and a reduction in chloroplast size.

Stages which are occasionally observed

Zoosporangia (see Fig. 3A, B, C). These may constitute up to 20 percent of the algal cells in some pellets. They are of the same dimensions as the other zooxanthellae and the cytoplasm contains inclusions, a pyrenoid and almost invariably an accumulation body. Each zoosporangium consists of a cyst wall enclosing a zoospore inside. This organism is released and becomes motile when the cyst wall ruptures (Fig. 3C).

Motile zoospores (see Figs. 3 D-F; 4 A-E). When present these cells may constitute up to 25 percent of the total algae in a pellet.

Structure

Zoospores are about the same size as vegetative cysts and ordinarily are somewhat dumb-bell shaped with no cyst walls. The wider anterior region (= the epicone) always contains a relatively large accumulation body and is separated from the narrower posterior region (= the hypocone) by a transverse groove which runs

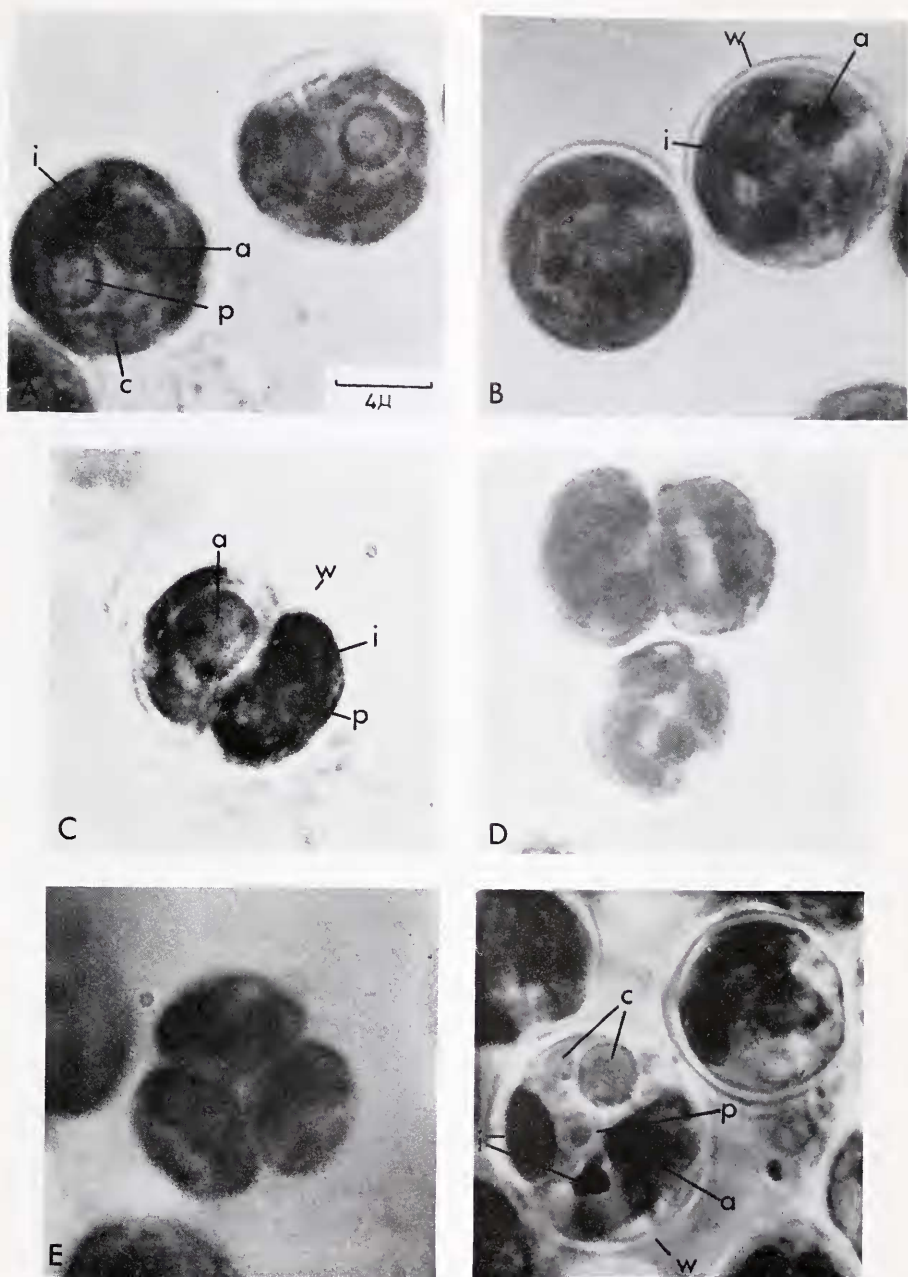
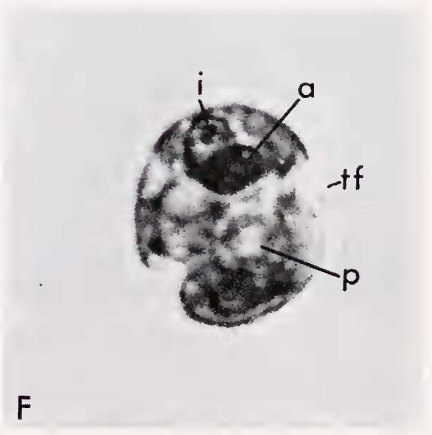
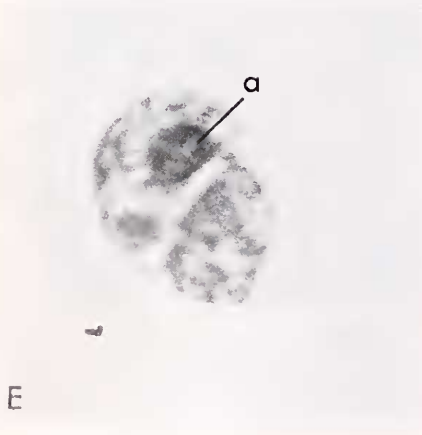
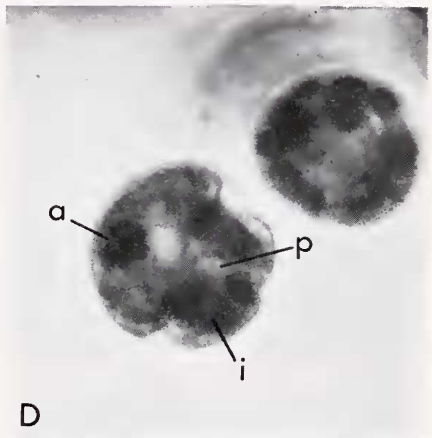
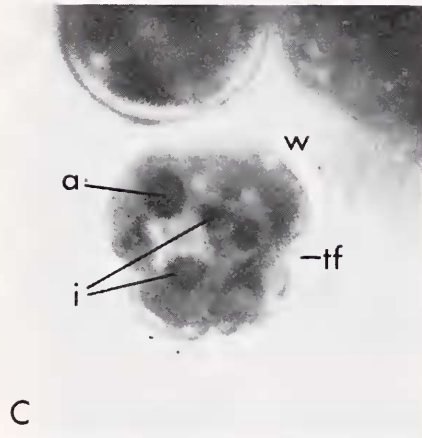
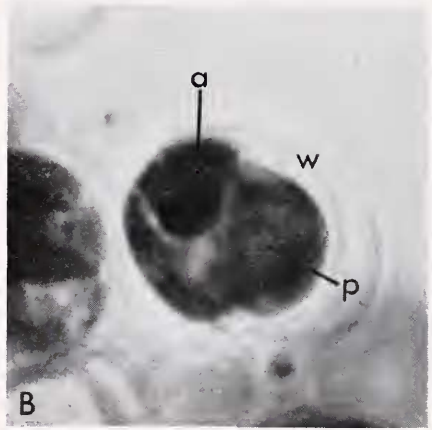
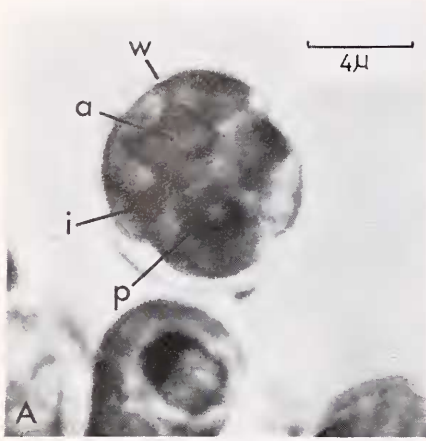


FIGURE 2. Photographs of various stages in the life history of the zooxanthella from *A. tagetes*. (A) shows immature cysts; (B) mature cysts (note cell walls); (C) a dividing cyst; (D) a dividing cyst in which the parent wall has been lost; (E) a cyst dividing into four daughter cells, and (F) degenerate cysts. Inclusions are represented by (i); other symbols are as in Figure 1. The 4 micron scale-bar in (A) is applicable to all six photographs.



around the organism and in which lies a flagellum. There is a depression at the tip of the posterior end which leads into a groove (= the sulcus) along one side of the organism which runs up to the transverse groove (see Fig. 3D). A long, posteriorly-directed flagellum extends from this groove and is about twice the length of the cell body (see Fig. 4C). All zoospores have more or less the same shape, but as they undergo contractions during swimming, there is variation from individual to individual.

Apart from the already mentioned accumulation body, chloroplasts and a pyrenoid are usually recognizable and in many cases other inclusions are also identifiable.

Locomotion

Two types of movements were displayed by these zoospores, each type alternating with the other at random intervals of time.

Forward movement (see Figs. 4C and 5). This type of movement is displayed when the organism darts rapidly from place to place with the anterior end foremost. Propulsion in these cases is supplied by the posterior flagellum and the organism rotates on the axis of this flagellum at the same time as it moves forward.

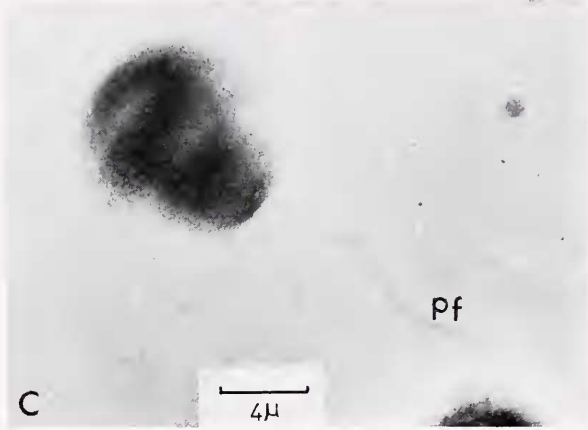
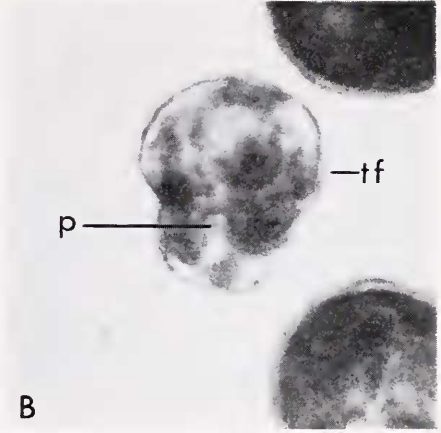
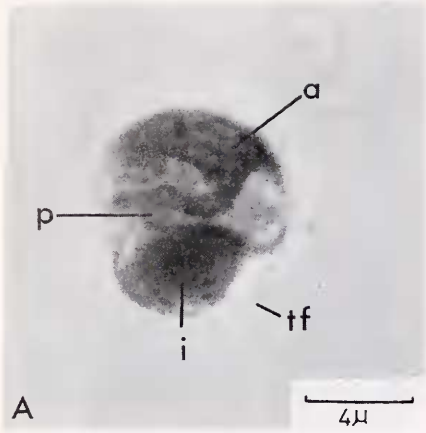
Gyratory movement (see Fig. 5). In order to carry out this type of movement, the zoospore first attaches itself to the substratum by means of the tip of the posterior flagellum. It then positions the cell body almost at right angles to the flagellum and gyrates. The gyrations are apparently produced through the action of the transverse flagellum. Individuals appear to gyrate to the right or to the left at random, and some have been observed to gyrate in one direction and then do so in the opposite direction immediately afterwards. The average rate of gyration is 3.3 revolutions per second at a room temperature of 21° C and zoospores have been observed to keep up one spell of gyrations for as much as 6½ minutes.

A number of these zoospores have been observed to stop swimming whereupon they immediately cast off their flagellae (see Fig. 4D and E) and become indistinguishable from the surrounding zooxanthellae.

DISCUSSION

The algal forms described in this paper are quite clearly stages in the life history of the zooxanthella symbiotic with *A. tagetes*. Figure 6 shows drawings of the identified stages arranged to show the likely relationship which they have with one another. Comparison of this outline with that of Taylor (1973a) (see Fig. 1) shows that probably only the sexual stages of the life cycle of this zooxanthella have not been found. Some of these algal stages resemble those of *S.* (= *G.*) *microadriaticum* shown in the photographs of McLaughlin and Zahl (1957) and Freudenthal (1962) as well as those shown in the diagram of the life cycle of *G. microadriaticum* outlined by Taylor (1973a). However, the zoospores

FIGURE 3. Photographs of various stages in the life history of the zooxanthella from *A. tagetes*. (A) and (B) show zoosporangia; (C) a zoospore breaking out of its cyst wall; (D), (E) and (F) zoospores. The transverse flagellum is represented by (tf); other symbols are as in Figures 1 and 2. The 4 micron scale-bar in (A) is applicable to all six photographs.



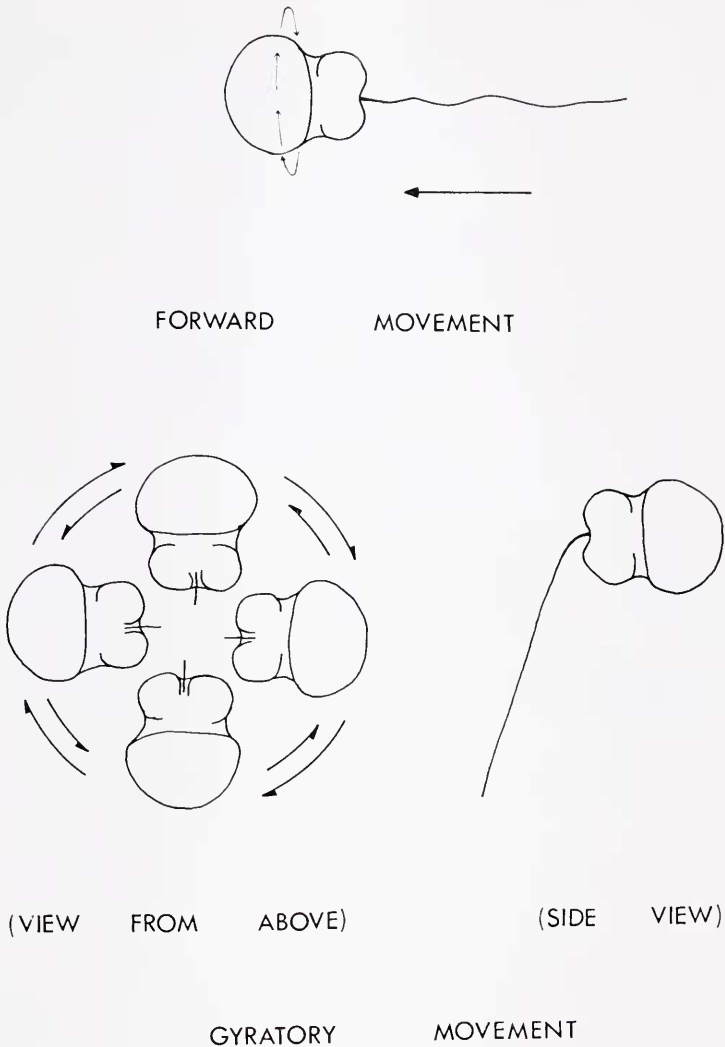


FIGURE 5. Diagrams showing the two types of movement carried out by zoospores of the zooxanthella of *A. tagetes*.

figured here resemble those obtained from *Acropora* by Kawaguti (1944), and differ from those described by McLaughlin and Zahl (1957) and Freudenthal (1962) in containing a relatively large reddish-brown accumulation body, which

FIGURE 4. Photographs of various stages in the life history of the zooxanthella from *A. tagetes*. (A) and (B) represent zoospores, showing the transverse flagellum; while (C) represents a swimming zoospore, showing the long posterior flagellum. The organism is rotating about the axis of this flagellum as it moves forward. (D) and (E) show successive stages in the release of its flagella by a zoospore which has just stopped swimming. The posterior flagellum is represented by (pf), other symbols are as in Figures 1-3. The 4 micron scale-bar in (A) is also applicable to (B) and (E); the 4 micron bar in (C) also to (D).

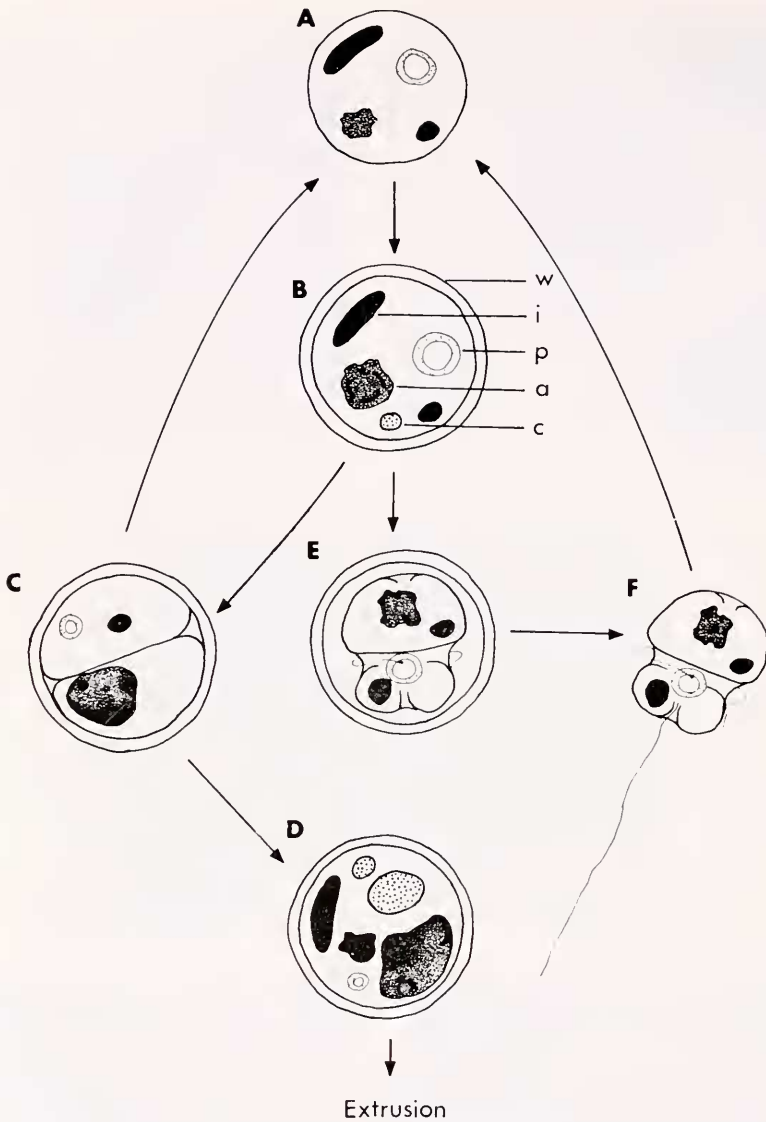


FIGURE 6. Outline of the likely relationships which exist between the stages in the life history of the symbiotic zooxanthellae from *A. tugetes* which have been identified in pellets extruded by the host. Chloroplasts are not drawn in. Symbols are as in preceding figures.

according to Taylor (1973b) is apparently a feature found only in the non-motile vegetative stages of dinoflagellate symbionts.

The irregular occurrence of an accumulation body in the zoospores referred to suggests either that the algae concerned belong to different strains—an idea for which there is some evidence (McLaughlin and Zahl, 1959; Trench, 1971a, b;

Kinzie, 1974)—or that cysts of any age may give rise to zoospores; young cysts thereby produce forms without accumulation bodies while older cysts which already possess such granules retain them when they undergo transformation into motile forms. Also, conceivable differences between the environments in which zoospores develop may cause the presence or absence of the accumulation body. Whether one or a combination of these is the correct answer is not known, but work on this problem is continuing.

The ejection of healthy symbiotic zooxanthellae by their hosts has been observed to occur as a normal process in *Zoanthus danae* (Le Conte) and *Zoanthus sp.* (Reimer, 1971), in *Palythoa sp.* (Trench, 1974) and in *A. tagetes* (Steele, present communication); and these new observations of motile zoospores in pellets extruded by *A. tagetes* serve to further underline the point that coelenterate hosts may release viable symbionts in this way. In addition, the fact that the motile forms have been found outside the host animal raises for the first time the definite possibility that under normal conditions a symbiotic zooxanthella could be transmitted from one host coelenterate to another by means of a motile stage in the alga's life history. Kinzie (1974), using cultures of dinoflagellates from various hosts, demonstrated that aposymbiotic specimens of the gorgonian *Pseudopterogorgia bipinnata* (Verrill) can be infected only by the zoospores of *G. microadriaticum* and not by the cysts; and he has also shown that during the process of infection the motile algae were attracted by and swam towards the gorgonian polyps.

On the basis of Kinzie's evidence, therefore, it seems very likely that zoospores released by *A. tagetes* would swim towards and infect any appropriate host which happened to be available. However, it must be pointed out that the existence of an indirect route for the transmission of symbionts, in which the released zooxanthellae are first engulfed by small organisms which in turn are captured and eaten by the host animal, is a distinct possibility. Evidence for this second route is to be found in the observation that zooxanthellae extruded by *A. tagetes* are frequently engulfed by ciliates of various kinds which are present in the pellet (R. D. Steele, unpublished observation).

The observed metamorphosis of zoospores into the vegetative cells takes place very rapidly, beginning in each case with the loss of the flagella followed by rounding up of the cell body. This process has also been described in *G. microadriaticum* both by McLaughlin and Zahl (1957) and Freudenthal (1962) and is obviously the norm among symbiotic zooxanthellae. The presence of behavior of this type in these motile forms suggests that the zoospore is definitely the dispersal stage of the alga whose mobility is lost once the process of transmission to another host has been accomplished. As to why both zoosporangia and zoospores are present in the extruded pellets of *A. tagetes* on some occasions only is not known, but work is continuing on this aspect of the subject.

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SUMMARY

1. Various morphological stages in the life history of the zooxanthella symbiotic with *Aiptasia tagetes* have been identified in pellets extruded by the actinian host. One of the stages is a motile zoospore.
2. This zoospore resembles that observed by Kawaguti (1944) and differs from those identified in axenic cultures of *G. microadriaticum* in containing a large accumulation body. Suggestions are made as to the significance of this difference.
3. It is very likely that the zoospore is an infective stage in the life history of the zooxanthella.
4. There is some evidence to suggest that indirect infection of hosts by zooxanthellae is also possible.

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